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β-AGAROFURAN SESQUITERPENES FROM *MAYTENUS CANARIENSIS*

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Key Word Index—*Maytenus canariensis*; Celastraceae; β-dihydroagarofuran sesquiterpenes.

Abstract—Two new polysterified sesquiterpenes with a dihydro-β-agarofuran skeleton were isolated and identified from data obtained by spectroscopy and selective hydrolysis.

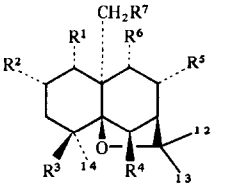
INTRODUCTION

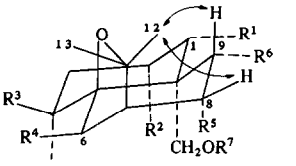
Maytenus canariensis (Loes.) Kunk. et Sund. [1] is a species used in popular medicine from which triterpenes, triterpene quinones [2, 3], the latter with antitumoural properties [4] and sesquiterpenes [5] have already been isolated. Chemotaxonomical evidence to connect this plant to the monospecies, *Catha edulis* Forsk, has also been obtained [6]. A further study of the minor constituents of the aerial part of *M. canariensis* has yielded compounds not obtained earlier, i.e. betulin, betulinic acid, the sesquiterpene lactone vulgarin (**14**) and the sesquiterpenes, triptofordin D-2 (**5**) [7], found previously in *Tripterygium wilfordii* H., **1** and **4**.

RESULTS AND DISCUSSION

Earlier papers reported [5] the isolation of new sesquiterpenes, **9** and **10**, with polyhydroxy isoalatol (**11**) [8] and 4β-hydroxyalatol (**12**) skeletons [8]. The sesquiterpenes now isolated have the same polyhydroxy skeletons and are esterified by acetic, benzoic, cinnamic and 2-methylbutyric acids.

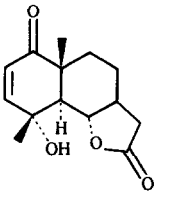
The formula $C_{33}H_{44}O_{12}$ and the structure 9α-benzoyloxy-8α-2-methylbutyroyloxy-1α,6β,15-triacetoxy, 4β-hydroxydihydro-β-agarofuran were assigned to compound **1** from the following: IR absorption bands at 3650 (OH), 1730 and 1710 (COO) cm^{-1}; a benzoate chromophore in the UV spectrum [9] which was confirmed by the loss of *m/z* 122 (C_6H_5COOH) and signals for five protons between δ 7.48 and 8.23 in the ^1H NMR spectrum; a mass spectral fragment at *m/z* 102 consistent with the presence of a 2-methylbutyric grouping [10] which is confirmed in the ^1H NMR by the signals at δ 0.80 (3H, *t*, $J = 8.0$ Hz) and 0.98 (3H, *d*, $J = 7.1$ Hz). Signals for three acetate methyls as singlets at δ 1.45, 2.12 and 2.26 confirm the presence of these groups as do the mass spectral fragments corresponding to the successive loss of *m/z* 60 (Me–COOH) and 42 ($CO{=}CH_2$). The compound was unaltered when treated with acetic anhydride in pyridine which, taken in conjunction with the IR spectral data and the presence of a one-proton singlet at δ 2.65 interchangeable with D_2O, indicates that the molecule contains a tertiary hydroxy group. Product **1** thus must have one benzoate, three acetates, a 2-methylbutyrate and a tertiary hydroxy. The ^1H and ^{13}C NMR data (Tables 1 and 2) are characteristic of a polysterified sesquiterpene with a dihydro-β-agarofurane skeleton [11].





NOE 1,4,5

	R¹	R²	R³	R⁴	R⁵	R⁶	R⁷
1	OAc	H	OH	OAc	OMeBut	OBz	OAc
2	OAc	H	OH	OH	OMeBut	OBz	OAc
3	OH	H	OH	OH	OMeBut	OBz	OH
4	OBz	H	OH	OAc	OMeBut	OBz	OAc
5	OCinn	H	OH	OAc	OAc	OBz	OAc
6	OCinn	H	OH	OH	OAc	OBz	OAc
7	OCinn	H	OH	OH	OH	OBz	OAc
8	OCinn	H	OH	OH	OH	OBz	OH
9	OBz	H	OH	OAc	OAc	OBz	OAc
10	OBz	OAc	OH	OAc	OAc	OBz	OAc
11	OH	H	OH	OH	OH	OH	OH
12	OH	OH	OH	OH	OH	OH	OH
13	OH	OH	H	OH	OH	OH	OH



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Double resonance studies and NOE experiments site ester groups on C-8 and C-9 with an α disposition since H-8 and H-9 shown a NOE when Me-12 is irradiated. H-9, H-8 and H-7 from an ABX system with signals centred at δ 5.68, 5.61 and 2.34, respectively, and with coupling constants (Table 1) identical to those of alatol (**13**) [12] and product **9** [5] previously isolated from the same

Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants (Hz)

	H-1	H-6	H-7	H-8	H-9	H-15	OAc-2	OAc-6	OAc-8	OAc-15
1	5.32	6.74	2.34	5.61	5.68	4.74, 4.91				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *dd*	1H, *d*	2H, d_{AB}	1.45	2.12		2.26
	4.5, 11.2		4.0	4.0, 5.8	5.8	13.4				
2	5.30	5.19	2.39	5.61	5.69	4.80, 4.94				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *dd*	1H, *d*	2H, d_{AB}	1.44			2.17
	4.5, 11.2		4.0	4.0, 5.8	5.8	13.4				
3	4.19	4.85	2.34	5.61	5.90	4.55				
	1H, *m*	1H, *d*	1H, *d*	1H, *dd*	1H, *d*	2H, m				
	$W_{1/2}$ 17.0	5.6	4.0	4.0, 5.8	5.8					
4	5.67	6.78	2.38	5.62	5.78	4.87, 5.07				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *dd*	1H, *d*	2H, d_{AB}		2.13		2.32
	4.5, 11,2		4.0	4.0, 5.8	5.8	13.4				
5	5.48	6.85	2.40	5.57	5.74	4.61, 5.16				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *dd*	1H, *d*	2H, d_{AB}		2.14	2.06	2.33
	5.1, 11.9		4.0	4.0, 5.4	5.4	13.3				
6	5.41	5.27	2.39	5.53	5.69	4.77, 4.95				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *dd*	1H, *d*	2H, d_{AB}			1.97	2.18
	5.1, 11.9		4.0	4.0, 5.4	5.4	13.3				
7	5.41	5.31	2.50	4.38	5.66	4.73, 5.08				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *m*	1H, *d*	2H, d_{AB}				2.24
	5.1, 11.9		4.0		5.4	13.3				
8	5.43	5.57	2.42	4.38	5.66	4.22, 4.46				
	1H, *dd*	1H, *br s*	1H, *d*	1H, *m*	1H, *d*	2H, d_{AB}				
	5.1, 11.9		4.0		5.4	13.3				

Table 2. ^{13}C NMR chemical shifts (δ, $CDCl_3$)

	1	**5**
1	75.3 *d*	75.4 *d*
2	25.2 *t*	25.2 *t*
3	37.9 *t*	38.0 *t*
4	70.7 *s*	70.7 *s*
5	92.2 *s*	92.2 *s*
6	73.3 *d*	72.8 *d*
7	53.4 *d*	53.3 *d*
8	78.3 *d*	78.3 *d*
9	69.7 *d*	70.4 *d*
10	52.2 *s*	52.5 *s*
11	82.9 *s*	82.8 *s*
12	24.4 *q*	24.4 *q*
13	29.5 *q*	29.6 *q*
14	22.6 *q*	22.8 *q*
15	60.9 *t*	60.7 *t*

plant. A proton which appears as a double doublet was assigned to the axial H-1β, as is standard in all dihydro-β-agarofuran skeleton sesquiterpenes isolated from the Celastraceae [11]. An AB system which signals centred at δ 4.74 and 4.91 was assigned to the protons of an ester-bearing methylene and was situated on C-15 from its chemical shifts [8]. The diamagentic shift to δ 1.45 of one of the acetate methyls indicates that one of the acetate groups and the benzoate should be in the positions 1 and 9 or *vice versa* [13], as often occurs in these sesquiterpenes.

The siting of the different esters was resolved as follows: selective hydrolysis of **1** with 0.1 M $NaHCO_3$ in methanol gave **2**, when **2** was treated with HCl (18%) in 1,4-dioxan, it gave **3**. The disposition of the ester groups in these compunds (Table 1) was unequivocally determined. Compound **2** was the 6-deacetyl-derivative of **1** and the ^{1}H NMR spectrum showed a shift of the H-6 signals from δ 6.74 in **1** to δ 5.19 in **2**. Compound **3** was the 1,6,15-trideacetyl-derivative of **1** as shown by the disappearance of the signals in the ^{1}H NMR spectrum for the three acetate methyls and the shift of their respective geminal protons (Table 1). As mentioned above, the chemical shift of the acetate on C-1 to δ 1.45 puts the benzoate on C-9 and, consequently, the 2-methylbutyrate must be on C-8. The data of compound **4** show it to be closely related to **1**:**4** has the formula $C_{38}H_{46}O_{12}$ and differs from **1** in that it has one acetate less and one benzoate more, the lost acetate being that on C-1 (Table 1). Its structure was thus established as 1α,9α-dibenzoylxy-8α-2-methylbutyroyl-oxy-6β,15-diacetoxy-4β-hydroxy-dihydro-β-agarofuran.

The structure of product **5** was established as triptofordin D-2 [7] from its spectroscopic data and those of its hydrolysis products, **6**–**8**. The spectral data for **6**–**8**, not previously given in the literature, are set out together with those of **5** for purposes of comparison.

The significance of the sesquiterpene eudesmanolide vulgarin (**14**) [14] being isolated for the first time from a Celastraceae together with β-agarofuran sesquiterpenes typical of this genus is still to be assessed. Other authors have already postulated the existence of a common precursor such as the germacrane cation [15].

EXPERIMENTAL

The plant was gathered at Icod, Tenerife and a voucher specimen is on file in the Departamento de Biología Vegetal, Facultad de Ciencias Biológicas, Universidad de La Laguna.

The aerial part of the plant (7 kg) was extracted with cold EtOH and 100 g of the extract was first chromatographed on Sephadex LH-20 with C_6H_{14}–$CHCl_3$–MeOH (2:1:1) as elutent, and the repeatedly on silica gel with C_6H_{14}–$CHCl_3$–MeOH and C_6H_{14}–EtOAc mixtures, to give β-amyrin, β-sitosterol, 3β,30-dihydroxy-lup-20-ene, betulin, betulinic acid, vulgarin, **1** (30 mg), **4** (12 mg) and **5** (42 mg).

9α-Benzoyloxy-8α,2-methylbutyroyloxy-1α,6β,15-triacetoxy-4β-hydroxydihydro-β-agarofuran (**1**). Amorphous solid, mp 57°, UV λ_{max}^{EtOH} nm: 286, 278, 234; $[M]^+$ at *m/z* 632.2745 (calc for $C_{33}H_{44}O_{12}$, 632.2737). IR ν_{max} cm^{-1}: 3650, 3010, 2900, 1730, 1710, 1580, 1350, 1210, 1070, 710. ^1H NMR (200 MHz) δ: 0.80 (3H, *t*, *J* = 9.0 Hz), 0.98 (3H, *d*, *J* = 7.1 Hz), 1.36 (3H, *s*), 1.56 (3H, *s*), 1.67 (3H, *s*), 2.67 (1H, *s*), 7.48 (3H, *m*), 8.23 (2H, *m*). EIMS *m/z* (rel. int.): 632 (1), 572 (2), 530 (5), 488 (6), 268 (2), 246 (8), 206 (8), 164 (16), 105 (100). Compound **1** (34 g), dissolved in MeOH (4 ml). was treated with 0.1 M $NaHCO_3$ (2 ml) under reflux for 6 hr at 50°, extracted and chromatographed to give **2**.

Compound **2**. Amorphous solid, mp 54°. IR ν_{max} cm^{-1}: 3540, 3010, 2900, 2850, 1750, 1370, 1280, 1100, 710. ^1H NMR (200 MHz) δ: 0.84 (3H, *t*, *J* = 9.0 Hz). 0.98 (3H, *d*, *J* = 7.1 Hz), 1.60 (3H, *s*), 1.67 (6H, *s*), 3.66 (1H, *s*), 7.41 (3H, *m*), 8.32 (2H, *m*); EIMS *m/z* (rel. int.): 572 $[M-18]^+$ (1), 512 (1), 488 (3), 468 (1), 428 (1), 366 (1), 351 (1), 330 (1), 306 (1), 105 (100). Compound **2** (20 mg) dissolved in dioxan (4 ml) was treated with HCl (15%) (4 ml) for 5 hr at 50°, affording **3**.

Compund **3**. Amorphous solid, mp 52°. IR ν_{max} cm^{-1}: 3540, 2950, 1750, 1280, 1100, 710. ^1H NMR (200 MHz) δ: 0.85 (3H, *t*, *J* = 9.0 Hz), 1.06 (3H, *d*, *J* = 7.1 Hz), 1.66 (3H, *s*), 1.72 (6H, *s*), 3.05 (1H, *br s*), 7.43 (3H, *m*), 8.00 (2H, *m*).

6β,15-Diacetoxy-1α,9α-dibenzoyloxy-8α-methylbutyroyloxy-4β-hydroxydihydro-β-agarofuran (**4**). Amorphous solid, mp 62°; UV λ_{max}^{EtOH} nm: 285, 278, 235; $[M-MeCOOH]^+$ at *m/z* 634.2747 (calc for $C_{36}H_{42}O_{10}$, 634.2755). IR ν_{max} cm^{-1}: 3540, 3000, 2950, 2910, 2850, 1750, 1450, 1370, 1280, 1240, 1100, 710. ^1H NMR (200 MHz) δ: 0.81 (3H, *t*, *J* = 9.0 Hz), 0.98 (3H, *d*, *J* = 7.1 Hz), 1.40 (3H, *s*), 1.58 (3H, *s*), 1.70 (3H, *s*), 6.88 (2H, *m*), 7.23 (2H, *m*), 7.35 (3H, *m*), 7.60 (3H, *m*). EIMS *m/z* (rel. int.): 634 $[M-60]^+$ (1), 592 (2), 550 (1), 428 (1), 306 (1), 268 (2), 246 (4), 105 (100).

Compound **5**. (38.7 mg), dissolved in MeOH (5 ml), was treated with 0.1 M $NaHCO_3$ (4 ml) for 4 hr at 50° and afforded **6**, **7** and **8**.

Compound **6**. Amorphous solid, mp 81°; $[\alpha]_D^{20}$ − 42.4°. UV λ_{max}^{EtOH} nm: 275, 231, 206. IR ν_{max} cm^{-1}: 3390, 3040, 2920, 2840, 1740, 1730, 1720, 1630, 1445, 1365, 1260, 1230, 710. ^1H NMR (200 MHz) δ: 1.56 (3H, *s*), 1.62 (3H, *s*), 1.64 (3H, *s*), 2.97 (1H, *s*), 5.63 (1H, *d*, *J* = 15.8 Hz), 6.85 (2H, *m*), 7.30 (7H, *m*), 7.85 (2H, *m*). EIMS *m/z* (rel. int.): 576 $[M-60]^+$ (2), 550 (1), 504 (1), 488 (1), 470 (1), 428 (2), 354 (3), 306 (2), 294 (2), 246 (10), 131 (43), 105 (100).

Compound **7**. Amorphous solid, mp 68°; $[\alpha]_D^{20}$ − 11.9°. UV λ_{max}^{EtOH} nm: 275, 230, 206. IR ν_{max} cm^{-1}: 3400, 2940, 2920, 2850, 1740, 1730, 1710, 1450, 1275, 1100, 855, 710. ^1H NMR (200 MHz) δ: 1.57 (6H, *s*), 1.60 (3H, *s*), 3.06 (1H, *s*), 5.68 (1H, *d*, *J* = 15.8 Hz), 6.90 (2H, *m*), 7.31 (7H, *m*), 7.85 (2H, *m*). EIMS *m/z* (rel. int.): 576 $[M-18]^+$ (1), 488 (4), 470 (1), 468 (2), 428 (2), 412 (2), 366 (3), 306 (2), 246 (10), 131 (16), 105 (100).

Compound **8**. Amorphous solid, mp 98°; $[\alpha]_D^{20}$ − 39.6°. UV λ_{max}^{EtOH} nm: 275, 230, 206. IR ν_{max} cm^{-1}: 3400, 2940, 2920, 2850, 1740, 1730, 1710, 1450, 1275, 1100, 710. ^1H NMR (200 MHz) δ: 1.58 (3H, *s*), 1.60 (3H, *s*), 1.73 (3H, *s*), 3.18 (1H, *s*), 5.69 (1H, *d*, *J* = 15.8 Hz), 6.90 (2H, *m*), 7.30 (7H, *m*), 7.85 (2H, *m*). EIMS *m/z* (rel. int.): 552 $[M]^+$ (1), 534 (1), 516 (1), 464 (1), 430 (1), 404 (1), 386 (11), 368 (3), 246 (8), 131 (66), 105 (100).

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